Eastern Regional Research Laboratory

Philadelphia, Pennsylvania

# TENTATIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF RUTIN IN VARIOUS PREPARATIONS

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## I. Scope

The methods outlined are intended for the determination of rutin in crude and purified rutin preparations and in pharmaceutical tablets, and the determination of quercetin and pigment impurities in such materials.

## II. Equipment and Materials

(1) Beckman ultraviolet spectrophotometer with matched 1 cm. absorption cells. Wave length must be checked and adjusted so that wave length scale errors are less than 0.2 mμ near 375 mμ. This should be done with a mercury lamp and quartz lens, using narrow slits and several mercury lines from 302.1 to 366.3 mμ.

(2) General Electric recording spectrophotometer for the visible spectrum (alternate: Beckman spectrophotometer with absorption cell compartment for 10 cm. cells), with matched 5 cm. cells.

(3) Modified Alber charging tube with attached capillary tube and removable ground glass cap (Fig. 1), for preparing and weighing anhydrous rutin samples.

(4) Vacuum drying oven, with drying agents.

(5) Drying oven for drying at 110° C.

(6) Volumetric flasks (100-ml.) and pipet (25-ml.).

(7) 95% ethanol.

(8) Absolute ethanol.

(9) Standard rutin preparation. Extract 5 - 10 grams of a purified rutin with benzene for 16 - 18 hours in a continuous extractor. Remove excess benzene with air and dry at 110° C. to remove as much of the benzene as possible from the extracted rutin. Crystallize from water, using silica gel. After recrystallization is complete, filter and dry the sample at 110° C. Dissolve in boiling 99% isopropanol in the proportion 7 grams per 100 ml. of isopropanol, concentrate to one-half volume, cool to room temperature, filter through an efficient heavy mat filter paper and pour into ten volumes of hot water. After crystallization is complete, filter and recrystallize once more from water without drying the rutin. The final crystals should be dried to constant weight at 110° C. Grind and store the preparation in a dark bottle in a dark place.

(10) Standard quercetin preparation. Prepare according to C. A. Morrow, "Biochemical Laboratory Methods for Students of the Biological Sciences," page 323, /John Wiley and Sons, New York (1927 Edition)/.

## III. Crude and Purified Rutin

(1) Proximate analysis for pigments. Dry the rutin preparation in air at 35° and then at 110° C. Weigh out about 200 mg. to the nearest mg.; dissolve in about 20 ml. of absolute ethanol; centrifuge the solution, and make to a volume of 50 ml. Examine in the visible spectrum for red pigment absorption maximum near 590 mμ and chlorophyll maximum near 655 mμ, using matched 5-cm. cells containing solution and solvent. Measure spectral densities at wave lengths 560, 590, 620, 655 and 690 mμ (or at the red pigment maximum and 30 mμ on each side and at the chlorophyll maximum and 35 mμ on each side). Calculate specific extinction coefficients at these wave lengths. Calculate the approximate proportions of red pigment and of chlorophyll in the preparation, using the equations shown below.

T = transmittance of solution relative to solvent
D = $\log 1/T$ = spectral density
k = D/bc = specific extinction coefficient
b = cell length in cm.
c = concentration of solution in grams/liter
$p = 4\left[k_{590} - \frac{1}{2}(k_{560} + k_{620})\right]$ = % red pigment
$c = k_{655} - \frac{1}{2}(k_{620} + k_{690})$ = % chlorophyll

If p is less than 0.0005% and c is less than 0.004%, the preparation is regarded as essentially free of pigment impurities. These limits are purely arbitrary and must be considered as tentative. See Fig. 2 for absorption curves for: A, a highly purified rutin preparation for which p = 0.0000% and c = 0.000%; B, a typical crude rutin; and C, a concentrate containing pigments from buckwheat leaves.

(2) Analysis for rutin and quercetin. After the preparation has been dried in air at 100° C., as in paragraph (1) above, place a sample of about 20 mg. size in the charging tube (Fig. 1). With the cap removed, continue drying for 16 hours at 130° C. in vacuo (pressure less than 3 mm. Hg) in the presence of magnesium perchlorate or barium oxide. At the end of this period, relieve the oven vacuum with air drawn through concentrated sulfuric acid and Drierite. Replace the cap on the drying tube as quickly as possible. Cool the tube to room temperature on a brass cooling plate. Weigh the dried sample, by difference, to the nearest 0.02 mg., transferring the sample to a 100-ml. volumetric flask. Add about 5 ml. of absolute ethanol, dissolve with warming if necessary, and finally make the solution to volume with 95% ethanol. Make a 16-fold dilution with 95% ethanol, using 100-ml. volumetric flasks and a 25-ml. pipet. Add 1 ml. of 0.02 N acetic acid to the final dilution before it is made to volume.

Using matched 1-cm. cells containing solution and solvent (also containing acid), measure spectral densities with great care at wave lengths 362.5 and 375.0 mμ. Calculate the density ratio $D_{375.0}/D_{362.5}$. If this ratio is 0.875 ±0.004 the preparation is free of quercetin, and the percentage of rutin in the sample is

$$r = 100\, k_{362.5}/33.22$$

Where $k_{362.5}$ is the specific extinction coefficient of the sample at 362.5 mμ and 33.22 is the coefficient for highly purified rutin under the conditions stated. The value of r is believed reliable to about ±0.5%.

If the density ratio is higher than 0.879, an appreciable amount of quercetin may be present. Calculate the specific extinction coefficients at 362.5 and 375.0 mμ, and compute the percentages of rutin and quercetin in the preparation by the equations:

$$r = 14.269\ k_{362.5} - 12.878\ k_{375.0} = \%\ \text{rutin}$$

$$q = -5.210\ k_{362.5} + 5.952\ k_{375.0} = \%\ \text{quercetin}$$

The estimated uncertainty is about ±0.7 both in percentage of rutin and of quercetin, provided errors in density and wave length are small.

The procedure should be run in duplicate.

IV. Pharmaceutical Preparations

This procedure is applicable to tablets containing 20 mg. of rutin with an excipient of lactose, gelatin, and calcium sulfate (or other materials having ultraviolet absorption negligible in comparison with that of the rutin present).

Weigh to the nearest 0.2 mg. a representative sample consisting of 10 tablets. Grind in a mortar, place the powder in a weighing bottle, and weigh (by difference) to the nearest 0.2 mg. a sample containing approximately 20 mg. of rutin. Transfer to a 60-ml. centrifuge tube, add 0.5 ml. of water, and stir for a few minutes. Add 25 ml. of 95% ethanol and dissolve the rutin by warming in a water bath. Centrifuge, then wash the insolubles twice by centrifugation, using 25-ml. portions of hot 95% ethanol to which 0.5 ml. of water has been added. Transfer the extract and combined washings to a 100 ml. volumetric flask, cool, and make to volume with 95% ethanol. Dilute 16-fold with 95% ethanol for ultraviolet spectrophotometric examination, adding 1 ml. of 0.02 N acetic acid to the final dilution before making to volume.

Measure spectral densities at 362.5 and 375.0 mμ. Calculate the density ratio $D_{375.0}/D_{362.5}$ and the specific extinction coefficients at the two wave lengths. (The concentration c grams/liter is 10 times the weight of the sample taken after grinding, divided by the dilution factor 16.)

If the density ratio is 0.875 ±0.004, the sample is essentially free of quercetin. The anhydrous rutin per tablet, in milligrams, is then:

$$r = W\ k_{362.5}/33.22 = \text{mg. anhydrous rutin}$$

where W is the weight of the 10 tablets in mg. divided by 10.

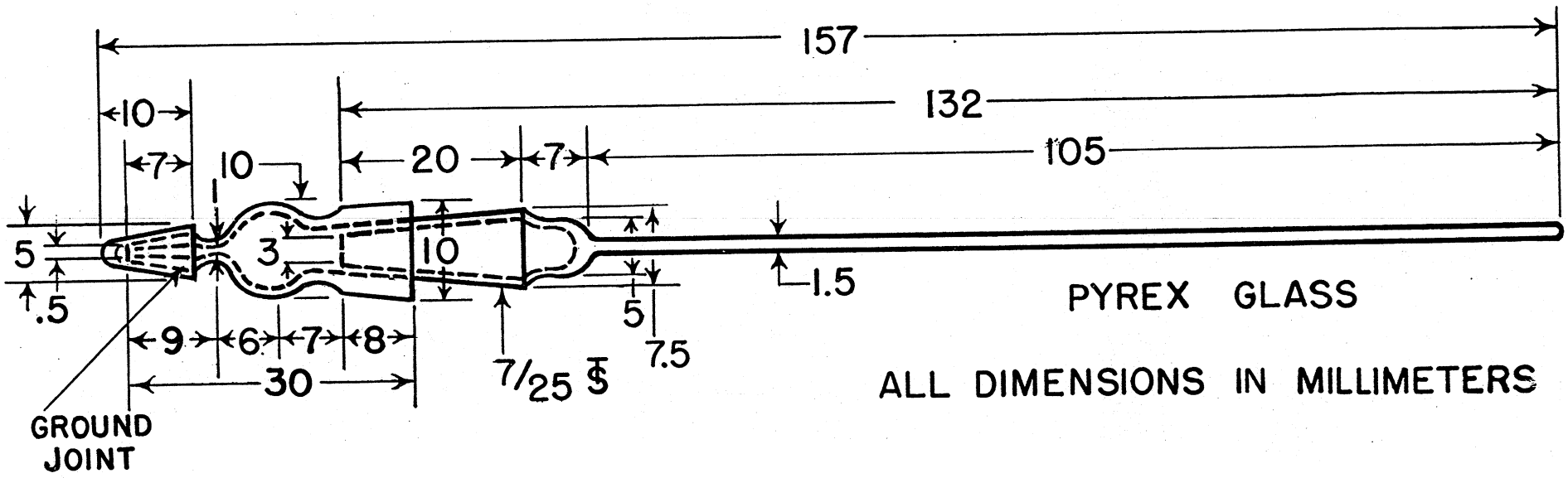
If the density ratio is greater than 0.879, an appreciable amount of quercetin is assumed to be present. In this case:

$$r = W(0.14269\ k_{362.5} - 0.12878\ k_{375.0}) = \text{mg. anhydrous rutin}$$

where W has the same meaning as before.

The reproducibility of the method, as tested by the average deviation of quadruplicate analyses on a composite sample of 10 ground tablets, is ±0.07 mg.

If dicalcium phosphate is used in the excipient, add 15 ml. of 0.02 N acetic acid in 95% ethanol to the dry powder followed by 10 ml. of 95% ethanol. Continue as in the procedure described, but do not add more acetic acid to the final dilution.




SEMIMICRO CHARGING TUBE

FIG. 1

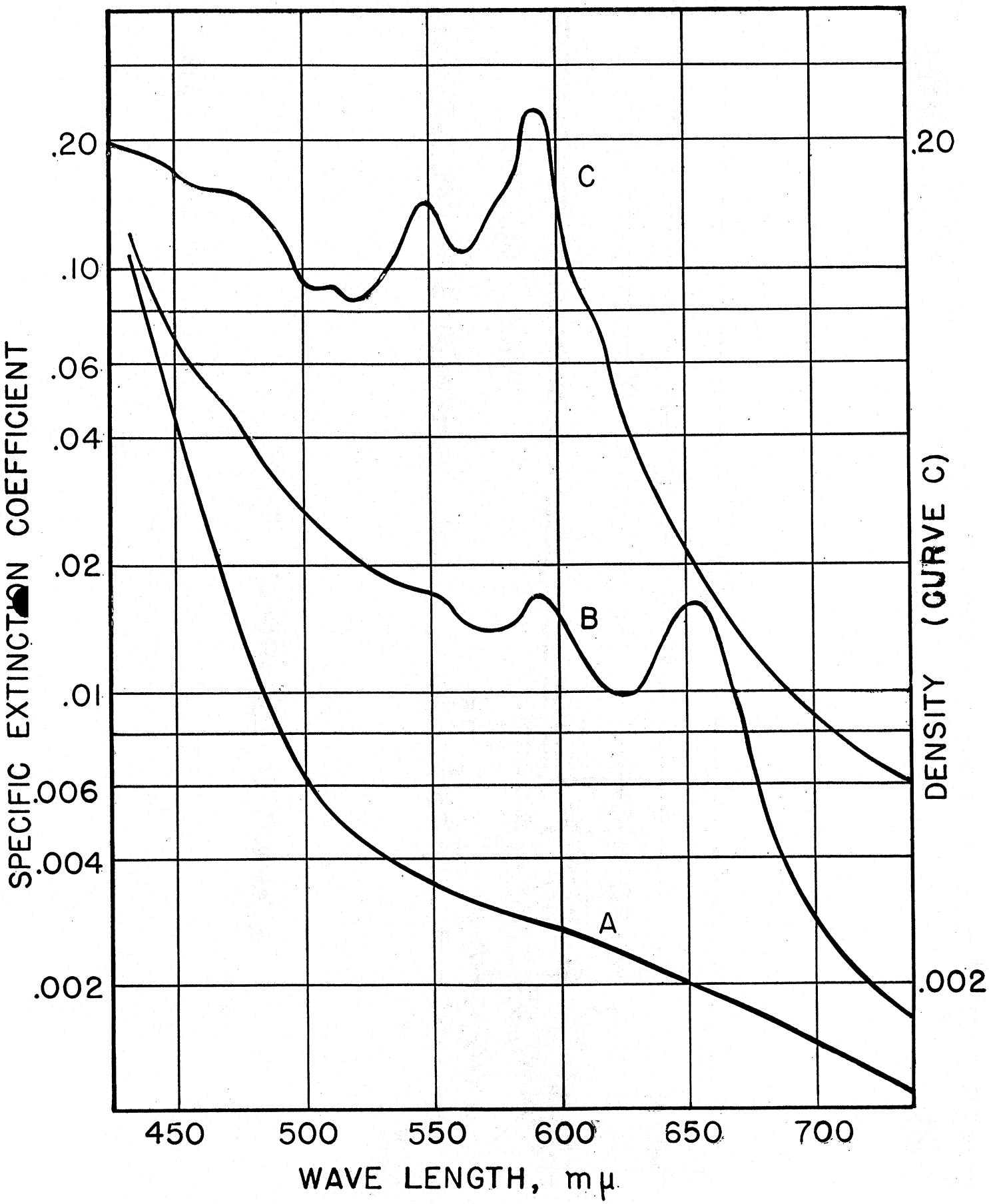

.20
.10
.06
.04
.02
.01
.006
.004
.002
SPECIFIC EXTINCTION COEFFICIENT
.20
.002
DENSITY (CURVE C)
C
B
A
450
500
550
600
650
700
WAVE LENGTH, mμ

FIG. 2

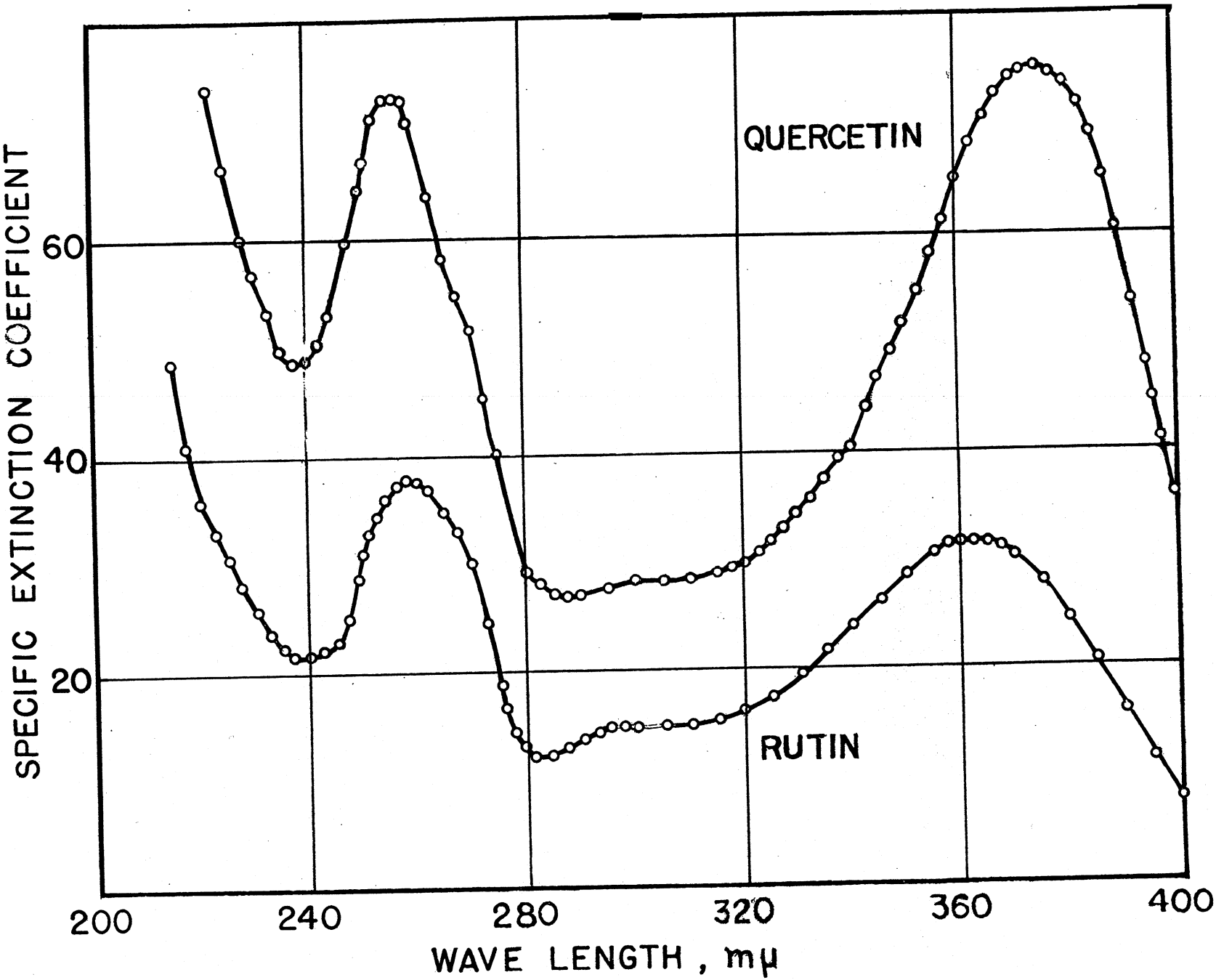

QUERCETIN
RUTIN
SPECIFIC EXTINCTION COEFFICIENT
60
40
20
200
240
280
320
360
400
WAVE LENGTH , mμ

FIG. 3